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(54) Title: NEUROTROPHIN ANTAGONISTS

(57) Abstract

A novel neurotrophin antagonist is described. The antagonist is a bicyclic peptide preferably derived from the internal reverse turn region of a member of the structurally similar neurotrophin family including NGF, BDNF, NT-3, NT-4, NT-5 and NT-6. Generally, the antagonist comprises amino acids from positions (58-68) and (108-110) of a neurotrophin, in which the amino acid from position 58 is covalently bound to the amino acid from position 108 and the amino acid from position 68 is covalently bound to the amino acid at position 110 to form a bicyclic structure. The neurotrophin-derived antagonists are useful to inhibit undesirable neurotrophin-mediated activity such as the neurite outgrowth that occurs in some neurodegenerative disease states.

LINEAR NGF PEPTIDE:

C^{68}-G-S-E-V-P-N-S-A-R-C^{58}-C^{108}-V-C^{110}

(SEQ ID NO:1)

CYCLIC NGF PEPTIDE:

C^{68}-G-S-E-V-P-N-S-A-R-C^{58}-C^{108}-V-C^{110}

(SEQ ID NO:1)

BYCYCLIC NGF PEPTIDE:

C^{68}-G-S-E-V-P-N-S-A-R-C^{58}-C^{108}-V-C^{110}

(SEQ ID NO:1)

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NEUROTROPHIN ANTAGONISTS

FIELD OF THE INVENTION

The present invention relates to neurotrophin antagonists. In particular, the present invention relates to neurotrophin-derived peptides which inhibit or reduce undesirable neurotrophin activity.

BACKGROUND OF THE INVENTION

A family of structurally and functionally related neurotrophic factors exist which are collectively known as neurotrophins. The family of neurotrophins include the nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4) neurotrophin-5 (NT-5) and neurotrophin-6 (NT-6).

The neurotrophins exhibit similar structural conformations, including three surface ß-hairpin loops, a ß-strand, an internal reverse turn region, and N- and C-termini. With respect to sequence similarities, the neurotrophins share approximately 50% amino acid identity. The neurotrophins are also functionally similar in that they each exhibit low affinity binding to a receptor known as the "p75 nerve growth factor receptor" or $p75^{NGFR}$. Each neurotrophin also exhibits binding to a receptor of the tyrosine kinase (trk) family which is of higher affinity than the binding to the p75 receptor. This interaction is believed to be related to neuron survival, but is also involved with neuron differentiation including process formation. The Trk receptor-neurotrophin interaction has been found to be more selective than neurotrophin interaction with the $p75^{NGFR}$ receptor. In particular, NGF binds only a trk receptor known as the TrkA receptor, while BDNF, NT-4 and NT-5 exhibit exclusive binding to a TrkB receptor. NT-3 is less selective and, although it binds primarily with a TrkC receptor, it also exhibits some binding to the TrkA and TrkB receptors (Ibanez et al., EMBO J. 1993, 12:2281).

The neurotrophins function primarily to promote survival of certain classes of peripheral and central neurons both during development and following neuronal

damage. NGF, in particular, is involved with the development of neurons in the peripheral nervous system and supports neuronal survival, as well as enhancing and maintaining the differentiated state of neurons. However, in some neurological disease states, the neurotrophins may also support inappropriate neurite outgrowth thereby facilitating the progression of a disease condition. For example, neurotrophins promote the undesirable sprouting of hippocampal "mossy fibres". Such inappropriate sprouting of mossy fibres is a common accompaniment of epilepsy in humans. In other pathological states, such as Alzheimer's disease, aberrant process growth, known as dystrophic neurite formation, is a strong correlate of disease severity.

Thus, although the neurotrophins are essential for the normal development and growth of neurons, they may be detrimental under certain circumstances. In such instances, ligands capable of inhibiting or reducing selected neurotrophin-mediated activities would be desirable therapeutically to treat neurodegenerative disease and repair of nervous system injury.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide peptides capable of inhibiting, or at least reducing, undesirable neurotrophin-mediated activity.

Accordingly, in one of its aspects the present invention provides bicyclic neurotrophin-derived peptides, or functional equivalents thereof, which inhibit a neurotrophin-mediated activity.

Another aspect of the present invention provides a peptide comprising amino acids from positions 58-68 and 108-110 of a neurotrophin, or a functional equivalent thereof, wherein the amino acid from position 58 is covalently bound to the amino acid from position 108 and the amino acid from position 68 is covalently bound to the amino acid at position 110 to form a bicyclic structure.

In another aspect of the present invention, a composition is provided which

includes a carrier and a peptide comprising amino acids from positions 58-68 and 108-110 of a neurotrophin, or a functional equivalent thereof, wherein the amino acid from position 58 is covalently bound to the amino acid from position 108 and the amino acid from position 68 is covalently bound to the amino acid at position 110 to form a bicyclic structure.

In a further aspect of the present invention, there is provided a method for inhibiting a neurotrophin-mediated activity comprising the step of exposing neurons to a composition as described above including a bicyclic peptide in combination with a suitable carrier.

A further aspect of the present invention provides a method for inhibiting neurotrphin-mediated activity in a mammal comprising the step of administering to said mammal a therapeutically effective amount of a composition which includes a bicyclic neurotrophin-derived peptide in combination with a pharmaceutical carrier.

These and other aspects of the present invention will be described in detail by reference to the following figures in which:

BRIEF REFERENCE TO THE DRAWINGS

Figure 1 illustrates generally the structure of a neurotrophin;

Figure 2 illustrates linear, cyclic and bicyclic peptides prepared from the 68-58/108-110 region of NGF;

Figure 3 graphically illustrates the effect of the bicyclic NGF peptide of Fig. 2 on neurite outgrowth; and

Figure 4 illustrates the effects of the peptides of Fig. 2 on kindling-induced seizures.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to bicyclic neurotrophin-derived peptides, or functional equivalents thereof, which inhibit a neurotrophin-mediated activity.

As it is used herein, the term "neurotrophin" refers to neurotrophic factors that are structurally homologous to NGF, i.e. include three surface ß-hairpin loops, a ß-strand, an internal reverse turn region, and N- and C- termini as illustrated in Fig. 1, and which promote at least one of neuron survival and neuron differentiation, as determined using assays of conventional design such as the in vitro assay exemplified herein and described by Riopelle et al. in the Can. J. of Phys. and Pharm., 1982, 60:707. Mammalian nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4) and neurotrophin-5 (NT-5) are examples of neurotrophins.

The term "neurotrophin-derived" refers to peptides comprising an amino acid sequence native to a given mammalian neurotrophin.

"Functional equivalents" of neurotrophin-derived peptides in accordance with the present invention are peptides which differ from a neurotrophin-derived peptide, by deletion, replacement or modification of one or more of its amino acids, but which retains the activity of the neurotrophin-derived peptide, i.e. is capable of inhibiting a neurotrophin-mediated activity. Functional equivalents of a neurotrophin-derived peptide in accordance with the present invention may comprise, for example, conservative amino acid replacements of native amino acids, e.g. an amino acid of the neurotrophin-derived peptide may be replaced with an amino acid having a similar charge such as replacement of an arginine residue with a lysine residue. Alternatively, the neurotrophin-derived peptide may include derivatized internal or terminal amino acids, as discussed in more detail herein, to yield a peptide which retains the activity of the neurotrophin-derived peptide.

"Neurotrophin-mediated activity" is a biological activity that is normally promoted, either directly or indirectly, in the presence of a neurotrophin. Neurotrophin-mediated activities include, for example, neurotrophin binding to the $p75^{NGFR}$ receptor or neurotrophin binding to one of the trk receptors, neuron survival, neuron differentiation including neuron process formation and neurite outgrowth, and biochemical changes such as enzyme induction. A biological activity that is mediated

by a particular neurotrophin, e.g. NGF, is referred to herein by reference to that neurotrophin, e.g. NGF-mediated activity. To determine the ability of a bicyclic peptide, or a functional equivalent thereof, to inhibit a neurotrophin-mediated activity, conventional in vitro and in vivo assays can be used. For example, a receptor affinity cross-linking assay, such as the assay described in herein in Example 2, can be used to assess the extent to which a bicyclic peptide inhibits neurotrophin/receptor binding. Peptide inhibition of neurite survival and outgrowth can be determined using the in vitro assay described by Riopelle et al. in the Can. J. of Phys. and Pharm., 1982, 60:707, exemplified herein in Example 3, or using the in vivo kindling experiment described in Example 4.

The term "bicyclic" is used herein to refer to a peptide in which there exists two ring closures. The ring closures are formed by covalent linkages between amino acids in the peptide. A covalent linkage between two non-adjacent amino acids constitutes a ring closure, as does a second covalent linkage between a pair of adjacent amino acids which are already linked by a covalent peptide linkage. The covalent linkages forming the ring closures may be amide linkages, i.e. the linkage formed between a free amino on one amino acid and a free carboxyl of a second amino acid, or linkages formed between the side chains or "R" groups of amino acids in the peptides. Thus, bicyclic peptides in accordance with the present invention may be "true" bicyclic peptides, i.e. peptides cyclized by the formation of a peptide bond between the N-terminus and the C-terminus of the peptide, or they may be "depsi-bicyclic" peptides, i.e. peptides in which the terminal amino acids are covalently linked through their side chain moities.

In one aspect, the bicyclic peptide is consistent with the internal reverse turn region of the selected neurotrophin. The reverse turn region of a neurotrophin extends from the amino acid at position 58 to the amino acid at position 68, and includes also the region extending from the amino acid at position 108 to the amino acid at position 110, as illustrated in Figure 1. The "reverse turn" results from the dual linkage occurring in this region. The dual linkage includes a first covalent linkage between the amino acid at position 58 and the amino acid at position 108, and

a second covalent linkage between the amino acid at position 68 and the amino acid at position 110.

Depsi-bicyclic peptides in accordance with the present invention result from the formation of covalent linkages between the side chains of the amino acids from positions 58, 68, 108 and 110. Preferably, the amino acid residues from these positions have side chains that will readily react to form such covalent linkages. For example, cysteine residues are particularly suitable amino acids for this purpose since the free thiol R groups of cysteine residues readily oxidize to form covalent disulfide bridges. Alternatively, the R groups of the amino acids in these positions can be derivatized to yield groups, such as free thiol groups, which will readily react to form the desired covalent linkages. In another alternative, amino acids from positions 58 and 108, and positions 68 and 110, can be selected to have R groups, or derivatized to yield R groups, which will form amide linkages. Thus, for example, an amide linkage can be formed between the amino acids from positions 58 and 108 if the amino acid at one of these positions yields a free amino group, while the amino acid at the other position yields a free carboxyl group. Examples of amino acids which yield a free amino group suitable for the formation of an amide bond are lysine, asparagine and glutamine. Examples of amino acids which yield a free carboxyl group suitable for the formation of an amide bond are glutamic acid and aspartic acid.

In the case of depsi-bicyclic peptides, it will be appreciated that the N- and C-termini remain as free amino and free carboxyl residues, respectively, since it is the side chains of the terminal amino acids which are involved in the covalent cyclizing linkage. The free terminal amino and carboxyl groups may also be derivatized or altered without affecting the activity of the peptide as an inhibitor of a neurotrophin-mediated activity. For example, the termini may be derivatized to include a non-peptidic blocking group that will prevent potential degradation at the N- and C-terminal ends from occurring. Such non-peptidic groups include protecting groups such as those conventionally used in the art of peptide synthesis which will not adversely affect the in vitro and in vivo uses of the bicyclic peptide. For example, suitable non-peptidic N-terminal blocking groups can be introduced by alkylation or

acylation of the N-terminus. Examples of suitable N-terminal blocking groups include C_1-C_5 branched or unbranched alkyl groups, acyl groups such as formyl and acetyl groups, as well as substituted forms thereof. Amino acid analogues lacking the amino functionality are also useful to block the N-terminus. Suitable non-peptidic C-terminal blocking groups, in which the carboxyl group of the C-terminus may be either incorporated or not, include esters, ketones or amides. Ester or ketone-forming alkyl groups, particularly lower alkyl groups such as methyl, ethyl and propyl, and amide-forming amino groups such as primary amines ($-NH_2$), and mono- and di-alkylamino groups such as methylamino, ethylamino, dimethylamino, diethylamino, methylethylamino and the like are examples of C-terminal blocking groups. Amino acid analogues lacking the carboxyl functionality are also useful C-terminal blocking groups such as agmatine. Further, it will be appreciated that the free amino and carboxyl groups at the termini can be removed altogether from the bicyclic peptide to yield desamino and descarboxylated forms thereof without affect on peptide activity.

True bicyclic peptides are also peptides in accordance with the present invention. Such peptides result from the formation of a peptide linkage between the N-terminal amino group of the amino acid from position 68 and the C-terminal carboxyl group of the amino acid from position 110.

Bicyclic peptides in accordance with the present invention may be derived from any mammalian neurotrophin due to the highly homologous nature of neurotrophins among different species with regard to both conformation and amino acid sequence. In particular, the amino acid residues of neurotrophins at positions 58, 68 and 108-110 are conserved across species and have been found to play an important role in peptide conformation. The amino acid residues in positions 59-67 do not appear to be important for peptide conformation, and further have not been found to participate in NGF interactions (Drinkwater et al., J. Biol. Chem. 1993, 268(31):23202). The bicyclic peptides of the present invention, thus, may be derived from the 58-68/108-110 amino acid region of, for example, human, mouse or rat NGF. Likewise, the bicyclic peptide may be derived from the 58-68/108-110 region

of any mammalian BDNF or NT-3. The following general formula (I) defines the bicyclic peptides:

$$AA^{68}\text{-XX-}C^{58}\text{-}C^{108}\text{-V-}AA^{110} \quad \text{(SEQ ID NO:1)} \qquad \text{(I)}$$

wherein

AA^{68} is selected from cysteine, the desamino form thereof, and an N-terminally blocked cysteine;

AA^{110} is selected from cysteine, descarboxylate cysteine, and a C-terminally blocked cysteine; and

XX represents a peptide comprising from 1-10 amino acid residues.

In one aspect the bicyclic peptide has an amino acid sequence represented by formula (II):

$$AA^{68}\text{-G-S-}X_1\text{-V-P-N-}X_2\text{-}X_3\text{-R-}C^{58}\text{-}C^{108}\text{-V-}AA^{110} \quad \text{(SEQ ID NO:2)} \qquad \text{(II)}$$

wherein

AA^{68} and AA^{110} are as defined above;

X_1 is an acidic amino acid;

X_2 is selected from the group consisting of a non-polar amino acid and an uncharged polar amino acid; and

X_3 is selected from the group consisting of an acidic amino acid and a non-polar amino acid.

In this regard, acidic amino acid refers to an amino acid which is negatively charged at pH 6.0, a non-polar amino acid refers to an amino acid having a non-polar side-chain such as alanine, leucine, methionine and proline, and an uncharged polar amino acid refers to an amino acid having an uncharged polar side-chain such as glycine, serine, cysteine and asparagine.

In another aspect the bicyclic peptide has an amino acid sequence represented generally by formula (II):

AA^{68}-G-S-X_1-V-P-N-X_2-X_3-R-C^{58}-C^{108}-V-AA^{110} (SEQ ID NO:2) (II)

wherein

AA^{68} and AA^{110} are as defined above;

X_1 is selected from glutamic acid and aspartic acid;

X_2 is selected from proline and serine; and

X_3 is selected from aspartic acid and alanine.

Specific peptide sequences in accordance with the present invention derived from mouse and human neurotrophins include:

NGF_m	C^{68}-G-S-E-V-P-N-S-A-R-C^{58}-C^{108}-V-C^{110}	(SEQ ID NO:3)
NGF_{hum}	C^{68}-G-S-D-V-P-N-P-D-R-C^{58}-C^{108}-V-C^{110}	(SEQ ID NO:4)
$BDNF_m$	C^{68}-G-E-K-T-Y-C-M-P-N-C^{58}-C^{108}-V-C^{110}	(SEQ ID NO:5)
$BDNF_{hum}$	C^{68}-G-E-K-T-Y-G-M-P-N-C^{58}-C^{108}-V-C^{110}	(SEQ ID NO:6)
$NT\text{-}3_m$	C^{68}-G-N-K-V-P-R-A-E-K-C^{58}-C^{108}-V-C^{110}	(SEQ ID NO:7)
$NT\text{-}3_{hum}$	C^{68}-G-N-K-V-P-R-A-E-K-C^{58}-C^{108}-V-C^{110}	(SEQ ID NO:7)

In a specific embodiment of the present invention, a bicyclic peptide derived from the 58-68/108-110 region of mouse NGF, as illustrated in Figure 2, was prepared and found to inhibit rat NGF-mediated activity. In particular, and as set out in detail in the specific examples herein, the bicyclic peptide inhibited cross-linking of NGF to both the $p75^{NGFR}$ receptor and the trkA receptor, and inhibited NGF-mediated neurite outgrowth as determined both in vitro and in vivo.

The bicyclic peptides of the present invention may be readily prepared by standard, well-established solid-phase peptide synthesis (SPPS) as described by Stewart et al. in Solid Phase Peptide Synthesis, 2nd Edition, 1984, Pierce Chemical Company, Rockfor, Illinois; and as described by Bodanszky and Bodanszky in The Practice of Peptide Synthesis, 1984, Springer-Verlag, New York. At the outset, a suitably protected amino acid residue is attached through its carboxyl group to a

derivatized, insoluble polymeric support, such as cross-linked polystyrene or polyamide resin. "Suitably protected" refers to the presence of protecting groups on both the α-amino group of the amino acid, and on any side chain functional groups. Side chain protecting groups are generally stable to the solvents, reagents and reaction conditions used throughout the synthesis, and are removable under conditions which will not affect the final peptide product. Stepwise synthesis of the oligopeptide is carried out by the removal of the N-protecting group from the initial amino acid, and coupling thereto of the carboxyl end of the next amino acid in the sequence of the desired peptide. This amino acid is also suitably protected. The carboxyl of the incoming amino acid can be activated to react with the N-terminus of the support-bound amino acid by formation into a reactive group such as formation into a carbodiimide, a symmetric acid anhydride or an "active ester" group such as hydroxybenzotriazole or pentafluorophenyl esters.

Examples of solid phase peptide synthesis methods include the BOC method which utilizes tert-butyloxycarbonyl as the α-amino protecting group, and the FMOC method which utilizes 9-fluorenylmethyloxycarbonyl to protect the α-amino of the amino acid residues, both methods of which are well-known by those of skill in the art.

Incorporation of N- and/or C- protecting groups can also be achieved using protocols conventional to solid phase peptide synthesis methods. For incorporation of C-terminal protecting groups, for example, synthesis of the desired peptide is typically performed using, as solid phase, a supporting resin that has been chemically modified so that cleavage from the resin results in a peptide having the desired C-terminal protecting group. To provide peptides in which the C-terminus bears a primary amino protecting group, for instance, synthesis is performed using a p-methylbenzhydrylamine (MBHA) resin so that, when peptide synthesis is completed, treatment with hydrofluoric acid releases the desired C-terminally amidated peptide. Similarly, incorporation of an N-methylamine protecting group at the C-terminus is achieved using N-methylaminoethyl-derivatized DVB resin, which upon HF treatment releases peptide bearing an N-methylamidated C-terminus. Protection of the C-

terminus by esterification can also be achieved using conventional procedures. This entails use of resin/blocking group combination that permits release of side-chain protected peptide from the resin, to allow for subsequent reaction with the desired alcohol, to form the ester function. FMOC protecting groups, in combination with DVB resin derivatized with methoxyalkoxybenzyl alcohol or equivalent linker, can be used for this purpose, with cleavage from the support being effected by TFA in dicholoromethane. Esterification of the suitably activated carboxyl function e.g. with DCC, can then proceed by addition of the desired alcohol, followed by deprotection and isolation of the esterified peptide product.

Incorporation of N-terminal protecting groups can be achieved while the synthesized peptide is still attached to the resin, for instance by treatment with suitable anhydride and nitrile. To incorporate an acetyl protecting group at the N-terminus, for instance, the resin-coupled peptide can be treated with 20% acetic anhydride in acetonitrile. The N-protected peptide product can then be cleaved from the resin, deprotected and subsequently isolated.

Recombinant techniques, well-established in the art, can also be used to prepare peptides in accordance with the present invention. DNA encoding the desired peptide is prepared and inserted into an appropriate expression vector. The vector is used to transfect a suitable host organism for production of the peptide. Conventional techniques are then used to culture the host and to isolate the peptide product from the cell culture media.

To ensure that the peptide obtained from either chemical or biological synthetic techniques is the desired peptide, analysis of the peptide composition should be carried out. Such amino acid composition analysis may be conducted using high resolution mass spectrometry to determine the molecular weight of the peptide. Alternatively, the amino acid content of the peptide can be confirmed by hydrolyzing the peptide in aqueous acid, and separating, identifying and quantifying the components of the mixture using HPLC, or an amino acid analyzer. Protein sequenators, which sequentially degrade the peptide and identify the amino acids in

order, may also be used to determine definitely the sequence of the peptide.

Having confirmed the identity of the peptide in linear form, it is then cyclized to form an active bicyclic peptide in accordance with the present invention. Many techniques are available for appropriately cyclizing the peptide, and the protocol used will depend on the type of linkages used to form the bicyclic product. As outlined above, there are numerous covalent linkages which are suitable to cyclize the peptide including, for example, side chain linkages such as disulfide linkages and peptide or amide linkages. In one embodiment of the present invention, disulfide linkages were used to form a bicyclic peptide as illustrated in Fig. 2. The internal cysteine residues of the linear peptide were first protected in order to conduct the cyclization reactions in a stepwise fashion. The protected peptide was then air oxidized to allow a disulfide linkage to form between the terminal cysteine residues. Following this cyclization reaction, the protecting groups were removed from the internal cysteine residues, and the peptide was again subjected to oxidizing conditions to allow a disulfide linkage to form between the internal cysteine residues thereby resulting in the bicyclic peptide.

Prior to its use to inhibit neurotrophin-mediated activity, the bicyclic peptide is purified to remove contaminants which may adversely affect its activity. In this regard, it will be appreciated that strict standards of purity, such as those required for pharmaceutical compounds, may not be required for use of the present compounds in vitro. On the other hand, if a compound according to the present invention is to be used in a pharmaceutical sense, it must be purified so as to meet the standards set out by the appropriate regulatory agencies. Any one of a number of conventional purification procedures may be used to attain the required level of purity including, for example, reversed-phase high-pressure liquid chromatography (HPLC) using an alkylated silica column such as C_4-, C_8- or C_{18}- silica. A gradient mobile phase of increasing organic content is generally used to achieve purification, for example, acetonitrile in an aqueous buffer, usually containing a small amount of trifluoroacetic acid. Ion-exchange chromatography can also be used to separate peptides based on their charge.

Bicyclic peptides of the present invention are useful to inhibit or reduce undesirable neurotrophin activity both in vitro and in vivo. Thus, in another aspect of the invention, a composition comprising an effective amount of a neurotrophin-derived bicyclic peptide and a suitable carrier is provided. By "suitable carrier" is meant a carrier which admixes with the selected bicyclic peptide to yield a composition suitable for the application for which it is to be used. By "effective amount" is meant an amount of bicyclic peptide sufficient to inhibit an undesired neurotrophin-mediated activity by about 50% as determined using assays of conventional design such as those described herein in the specific examples.

The present bicyclic peptides have use as media supplements to prevent undesirable neurotrophin-mediated activity of neuron cells in vitro. For example, primary sensory neurons require NGF for survival in cell culture; however, NGF also influences neuron differentiation, notably process formation and outgrowth, which are undesirable for the use of primary sensory neurons in cell culture. Thus, to preserve neuron survival in vitro while inhibiting cell differentiation, NGF is added to the cell culture media along with a bicyclic peptide. For addition to the cell culture, the bicyclic peptide is first combined with a carrier which will not adversely affect the growth of the cells in culture. Such carriers will include, for example, physiologically acceptable fluids such as water or any other fluid suitable for addition to the cell culture. Alternatively, the peptide can be combined with media suitable for culturing neuronal cells prior to being added to the cell culture. To be effective to prevent neuron differentiation, the concentration of the peptide in the cell culture will be in the range of from about 1-500 μM, and preferably from about 1-100 μM. The optimal concentration of bicyclic peptide for use in preventing neuron differentiation in cell culture will, of course, vary in each independent case, and will depend on the extent of inhibition desired as well as the type of neuronal cells involved.

Compositions for in vivo administration, e.g. for treating neurological conditions such as epilepsy or Alzheimer's disease, are also contemplated. Such compositions comprise a therapeutically effective amount of a bicyclic peptide

together with a pharmaceutically acceptable carrier. In this context, the term "pharmaceutically acceptable" means acceptable for use in the pharmaceutical and veterinary arts, i.e. non-toxic and not adversely affecting the activity of the bicyclic peptide. The term "therapeutically effective amount" means an amount of the compound sufficient to reduce undesirable neurotrophin-mediated activity, as determined using assays of conventional design such as the assays described herein in the specific examples, in an inflicted individual without causing adverse effects.

Pharmaceutically acceptable carriers useful to prepare compositions for in vivo administration include conventional carriers generally selected for combination with peptide-based drugs such as diluents, excipients and the like. Reference may be made to "Remington's Pharmaceutical Sciences", 17th Ed., Mack Publishing Company, Easton, Penn., 1985, for guidance on drug formulations generally. As will be appreciated, the pharmaceutical carriers used to prepare compositions in accordance with the present invention will depend on the administrable form to be used to treat the inflicted individual.

According to one embodiment of the invention, the compounds are formulated for administration by injection intraventricularly, and are accordingly provided as aqueous solutions in sterile and pyrogen-free form and optionally buffered or made isotonic. Thus, the compounds may be administered in distilled water or, more desirably, in saline or 5% dextrose solution. Water solubility of these and other compounds of the invention may be enhanced, if desired, by incorporating into the composition a solubility enhancer, such as cetyltrimethylammonium bromide or chloride. Lyoprotectants, such as mannitol, sucrose or lactose and buffer systems, such as acetate, citrate and phosphate may also be included in the formulation, as may bulking agents such as serum albumin.

Alternatively, the compounds of the present invention may be formulated for administration by routes other than injection. For example, oral dosage forms, such as tablets, capsules and the like, formulated in accordance with standard

pharmaceutical practise, may be employed.

For use in treating individuals with a neurological condition, precise dosage sizes of a pharmaceutical composition appropriate for treatment can readily be established in appropriately controlled trials, and will correspond to an amount of bicyclic peptide that reduces undesirable neurotrophin-mediated activity without causing intolerable side effects to the individual being treated. It is anticipated that an effective treatment regimen for patients will involve the intraventricular administration of dosages which achieve a level of peptide in the spinal fluid of the individual being treated of about 1-500 μM. It will be appreciated, of course, that the dosage sizes required to attain this in vivo concentration will vary according to the route of administration, the frequency of administration, on the individual being treated and on the neurological condition being treated.

Specific embodiments of the present invention are described in more detail in the following examples which are not to be construed as limiting.

Example 1 - Synthesis of NGF Bicyclic Peptide

The bicyclic peptide illustrated in Fig. 2 was prepared by first synthesizing the linear form thereof using the solid phase synthesis method. An automated synthesizer, e.g. Applied Biosystems 430A, was used with a Wang resin (available from NovaBiochem). All amino acid side chains were protected with Mtr (4-methoxy-2,3,6-trimethyl-benzene-sulfonyl) groups, with the exception of the cysteines from positions 58 and 108 which were protected with ACM (acetamidomethyl) groups. A TFA-cleavage (1-2 hrs) from the resin yielded the linear peptide retaining only the ACM protecting groups.

To cyclize the peptide, the two free thiol groups at positions 68 and 110 were then covalently linked. The peptide was dissolved in 0.1 M ammonium bicarbonate buffer, pH 8.3, at a concentration of 0.1 mg/mL. The reaction mixture was stirred at room temperature, and the progression of the reaction was monitored by HPLC. The HPLC solvent, comprising solvent A of water with 0.1% TFA and solvent B of

acetonitrile with 0.1% TFA, was run on a gradient from solvent A to solvent B at 1% per minute. On completion of the reaction, the mono-cyclized product was isolated by lyophilization and purified by HPLC.

The mono-cyclized product (0.1 mmol) was then cyclized at the ACM protected sites. The product was dissolved in 1.5 mL of methanol. This solution was added dropwise to 2.5 mL of methanol containing 63.5 mg of iodine over 30 minutes with stirring at room temperature. The stirring was continued (approx. 3 hrs) while the progression of the reaction was determined using HPLC. Upon completion of the cyclization, the reaction was quenched by the addition of solid zinc powder (1-2 mg). The mixture was diluted with 10 mL of water, filtered and lyophilized. The bicyclic product was purified using HPLC, and its structure was confirmed by standard methods.

Example 2 - Affinity Cross-Linking Experiments

The ability of the peptides derived from the 68-58/108-110 region of NGF to antagonize NGF interaction with the p75 and trkA receptors was determined. The peptides tested were bicyclic (BC) 68-58/108-110, cyclic (C) 68-58/108-110, and linear (L) 68-58/108-110, each of which are illustrated in Figure 2.

PC12 rat pheochromocytoma cells (ATCC CRL 1721) were independently incubated in RPMI (GIBCO)/10% fetal calf serum (GIBCO) with 20 μM and 200 μM solutions of NGF peptide, prepared as described in detail in Example 1, in the presence of ^{125}I-NGF (isolated from mouse submaxillary gland as described in Mobley et al., 1976, Biochemistry, 15:1543) for 2 hours at 4°C. Control PC12 cells were incubated in RPMI/10% fetal calf serum in the presence of ^{125}I-NGF only (no NGF peptide).

For trkA cross-linking, bis-(sulfosuccinimidyl)suberate (Pierce) was added to the incubation mixture to a final concentration of 0.4 mM (in 20 μl), and incubated for 20 min at 25°C. For cross-linking to p75NGFR, N-hydroxysulfosuccinimide (Pierce) was added to the incubation mixture to a final concentration of 2 mM and 1-

ethyl-3-(3-dimethylaminopropyl)carbodiimide (Pierce) was added to a final concentration of 5 mM (in 20 μl), and incubated for 30 min at 25°C. On completion of the cross-linking reaction, the cells were washed three times in HKR buffer (Gibco) with BSA at 4°C to remove excess free radiolabelled ligand and reagents.

The cells were solubilized in 1 ml of lysis buffer containing 10 mM tris-HCl, pH 7.4, 150 mM NaCl, 10% glycerol, 1% NP-40, 0.01 mg/ml aprotinin, 0.5 mM orthovanadate, 0.5 μl/ml leupeptin, and 2 mM phenyl-methyl-sulfonylfluoride at 4 °C for 15 min. Cellular debris was removed by centrifugation. TrkA antibody (supplied by W. Mobley, University of California, San Francisco) or p75 antibody (supplied by E. Shooter, Stanford U., California) were added to the supernatant to a final concentration of 20 μg/ml, and incubated at 4°C for 2 hrs. with constant mixing. Protein A Sepharose (Sigma) pre-equilibrated in lysis buffer was added (50 μl of a 50% solution) to the TrkA sample and incubated at 4°C for 2 hrs. For the p75 sample, pre-equilibrated goat anti-mouse agarose (Sigma) was added (50 μl of a 50% solution) and incubated.

The immunoprecipitated proteins were then washed with lysis buffer and eluted from the affinity gels using reducing SDS sample buffer, and solubilized in the reducing buffer for 10 min at 95°C. Samples were separated on a modified Laemmli discontinuous acrylamide gel system (Laemmli, Nature, 1970, 227:680) using 4% SDS PAGE staking gel and a gradient urea polyacrylamide separating gel ranging from 4.5% acrylamide/18% urea to 7.5% acrylamide/48% urea. The gels were fixed and processed for autoradiography using -70°C exposure with Kodak XAR film and manual processing.

The following results were obtained by observing the density of bands (the less the density, the greater the antagonism) appearing on the autoradiograms:

PEPTIDE	Inhibition of P75 Interaction	Inhibition of TrkA Interaction
1. BC 20 μM 2. BC 200 μM	1. +[1] 2. ++[2]	1. ++ 2. ++
C 200 μM	no inhibition	no inhibition
L 200 μM	no inhibition	no inhibition

[1] "+" indicates less than 50% inhibition
[2] "++" indicates greater than 50% inhibition

Thus, as can be seen from the tabulated results, only the bicyclic peptide was capable of inhibiting NGF interaction at the p75 and TrkA receptors.

Example 3 - Inhibition of Neurite Outgrowth

Eight-day chick embryo dorsal root ganglia (DRG) were freed of meninges and removed aseptically. The DRG were kept at 4°C at all times. Ganglia from six embryos (40-50 per embryo) were washed in Ca^{2+}- and Mg_{2+}- free Gey's balanced salt solution (Gibco) and exposed to 0.01% trypsin (Worthington) in the same solution for 10 min at 37°C. A half-volume of phosphate-buffered Gey's balanced salt solution was added for a further 5 min at 37°C and the reaction was then stopped with one-third volume of Ham's F12 medium (Gibco) containing 5% fetal calf serum (Gibco). The ganglia were then triturated using a 5 mL narrow-tip pipette to a single cell suspension. Following filtration through 37-μm nylon mesh (Small Parts Inc., Miami, FL) in a millipore chamber to remove clumps, the cell suspension was washed through a 500-μl FCS undercut (700 x g for 5 min at 4°C) and resuspended in 4 mL of Ham's F12 medium plus 5% FCS. The cell suspension was then preplated on a 100-mm Flacon culture dish and incubated for 45-60 min at 37°C in a 5% CO_2 humidified atmosphere. Cells enriched in neurons were decanted for the bioassay, since non-neuronal cells of DRG preferentially stick to the culture substrate.

The inside wells of 96-well Falcon microculture plates were coated with polylysine (0.1 mg/mL) (Sigma) for 4 h at 37°C (the outside wells were filled with distilled water to provide humidity) and, following a rinse with tissue culture media, 100 μL of neuron-rich cell suspension was added to each well at 10^5 cells/mL.

Ninety (90) μL of NGF solution (prepared in tissue culture media) was then added to each well to a final concentration of 0.25 ng/mL NGF per well. Ten (10) μL of bicyclic 68-58/108-110 peptide solution, i.e. tissue culture media admixed with bicyclic peptide prepared as described in Example 1, was then added to test wells in duplicate to yield wells containing 0 μM, 25 μM, 100 μM and 250 μM peptide. For control assays, 10 μL of Ham's F12 medium was added to duplicate NGF-containing wells. The plates were covered and incubated in the dark for 24-30 hrs. at 37°C in a 5% CO_2 humidified atmosphere.

The bioassays were read using a Leitz Diavert microscope with phase optics. To afford adequate optics, the meniscus effect of each well was removed by filling the well with a balanced salt solution until a flat, air-filled interface was achieved at the top of the well. At least 100 neurons per well were counted, and the assay was scored as the ratio of cells bearing neurites greater than one cell diameter to total viable (phase-bright) cells.

The results of this assay are illustrated in Fig. 3. In this experiment the IC_{50}, i.e. the concentration of bicyclic peptide required to inhibit neurite growth on 50% of the cells, was calculated to be 250 μM.

Example 4 - Effect of Peptide on Neuron Survival

Cells enriched for sensory neurons were prepared from ED8 chick DRG as described above. The cells were plated at a density of 800-1000 cells per well in Terasaki plates treated with poly-D-lysine and laminin in tissue culture medium containing 1 ng/ml NGF and 68-58/108-110 depsibicyclic peptide in the amounts shown below. Following a 20-22 hr. incubation at 37°C, 5% CO_2, the cells were fixed in 4% formaldehyde in PBS and cells on the tissue culture surface were counted as a percentage of total cells.

Additives	% Viable Cells
0	78.2 ±1.1
methanol (1.8%)[1]	66.3 ±5.0
peptide (5 nM)	70.4 ±3.4
peptide (50 nM)	67.2 ±1.2
peptide (500 nM)	86.5 ±4.6
peptide (5 μM)	79.5 ±2.4
peptide (20 μM)[2]	79.5 ±1.4

[1] highest final concentration used as a vehicle for peptide
[2] This concentration is twice the IC_{50} for neurite growth inhibition.

As can be seen from the results of this assay, the depsibicyclic peptide had no significant effect on NGF-mediated survival at the concentrations tested.

Example 5 - Effect of Neurotrophin-Derived Peptide on Kindling

Kindling is a phenomenon in which repeated low-intensity (subconvulsive) electrical stimulation of forebrain areas leads to a progressive and permanent amplification of seizure activity, and is thus, widely accepted as a model for human temporal lobe epilepsy. The effect of the present neurotrophin-derived peptides on kindling was determined as follows.

Male Long-Evans hooded rats (300-400 g) were used. The animals were housed individually, maintained on an ad lib feeding schedule and kept on a 12 h on/12 h off light cycle.

The rats were anesthetised with 0.1 ml per 100 g body weight of 100 mg/ml ketamine hydrochloride (Rogar/STB Inc., London, Canada) and 0.05 ml per 100 g body weight of 20 mg/ml xylocaine 2% hydrochloride (Astra, Mississauga, Canada), and then placed in a stereotaxic holder. The rats were implanted unilaterally with a bipolar twisted, teflon-coated, stainless steel electrode with an exposed tip (wire diameter 190 μm) in the right amygdala at stereotaxic coordinates of 3.3 mm caudal and 8.0 mm lateral to bregma and 8.5 mm ventral to the brain surface (selected from Paxinos and Watson, 1982, "The rat brain in stereotaxic coordinates", Academic

Press, Sydney). Following implantation of the electrode, a cannula (Alzet brain infusion kit, Alza Corp.) was implanted in the lateral ventricle, 5 mm below the skull surface, at 0.6 mm caudal to bregma and 1.3 mm lateral to the midline. It was firmly attached to the skull with dental cement and anchored with three stainless steel screws. An osmotic pump (Alzet model 2002, flow speed 0.5 μl/h, effective maximally for 14 days) was connected to the cannula via polyethylene tubing and placed subcutaneously in the neck area. Histological examination of lateral ventricle sections was done to confirm that the cannula was correctly placed. Forty-five (45) μM of peptide, in a physiologically acceptable buffer, was delivered throughout the duration of the experiment to each test animal. There were five groups of test animals, 5 animals per peptide test group, 10 animals in the negative control group and 12 animals in the positive control group. Each test group was administered one peptide selected from the linear, cyclic and bicyclic 68-58/108-110 peptides. The negative control group was infused with control serum IgGs, and the positive control group was infused with 100 μg/day of anti-NGF antibody. The anti-NGF antibody was obtained from sheep immunized with 0.5 mg of 2.5S NGF (prepared from male mouse salivary glands according to the method of Mobley et al., *supra*) intradermally in complete Freund's adjuvant initially, and in incomplete adjuvant every 4 weeks thereafter. Blood was collected 10 days after each booster injection. Serum was prepared by clotting the blood at room temperature followed by centrifugation at 1,500 g for 30 min., heat inactivation at 56°C for 30 min. and sterilization using 0.22 μm filters (Nalgene). IgG was purified from serum by differential precipitation using caprilic acid followed by ammonium sulfate (McKinney and Parkinson, 1987, J. Immunol. Methods, 96:271). NGF-specific antibody was further purified using affinity chromatography on 2.5S NGF coupled to CN-Br sepharose 4B (Pharmacia).

Following a three-day recovery, the kindling stimulations were started. The animals received a one-second train of one-millisecond pulses at a frequency of 60 Hz and a pulse intensity of 200-400 μA. These pulses were sufficient to trigger an epileptiform afterdischarge (AD) following each stimulation. Each animal was stimulated in this fashion twice a day over a period of 11 days. Progression of kindling was monitored behaviorally and electrophysiologically by recording the

behavioral seizure stages and the duration and magnitude of afterdischarges. Fully kindled animals exhibited three consecutive stage-5 seizures (Racine, 1972, Electroencephalogr. Clin. Neurophysiol., 32:281).

The number of stimulations to reach stage-5 seizures for control rats and rats receiving the linear, cyclic and bicyclic peptides is illustrated graphically in Fig. 4. The results illustrate that the bicyclic peptide has a potency which is approximately equal to that of the anti-NGF IgG in delaying the onset of kindling in comparison to the control serum IgG, linear peptide and cyclic peptide.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Riopelle, Richard J.

(ii) TITLE OF INVENTION: NEUROTROPHIN ANTAGONISTS

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Ridout & Maybee
(B) STREET: 2300 Richmond-Adelaide Centre, 101 Richmond Street West
(C) CITY: Toronto
(D) STATE: Ontario
(E) COUNTRY: Canada
(F) ZIP: M5H 2J7

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (416) 868-1482
(B) TELEFAX: (416) 362-0823

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Xaa at pos.1 is Cys, desamino Cys or N-terminally blocked Cys"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Xaa at pos.2 represents from 1-10 amino acid residues"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "Xaa at pos. 6 is Cys, decarboxylated Cys or N-terminally blocked Cys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa Xaa Cys Cys Val Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Xaa at pos.4 is an acidic amino acid"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Xaa at pos.1 is Cys, desamino Cys or N-terminally blocked Cys"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION: /note= "Xaa at pos.8 is a non-polar amino acid or an uncharged polar amino acid"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= "Xaa at pos.9 is an acidic amino acid or a non-polar amino acid"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 14
(D) OTHER INFORMATION: /note= "Xaa at pos.14 is Cys, descarboxylated Cys or a C-terminally blocked Cys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Gly Ser Xaa Val Pro Asn Xaa Xaa Arg Cys Cys Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Cys Gly Ser Glu Val Pro Asn Ser Ala Arg Cys Cys Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Gly Ser Asp Val Pro Asn Pro Asp Arg Cys Cys Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Cys Gly Glu Lys Thr Tyr Cys Met Pro Asn Cys Cys Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Cys Gly Glu Lys Thr Tyr Gly Met Pro Asn Cys Cys Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Cys Gly Asn Lys Val Pro Arg Ala Glu Lys Cys Cys Val Cys
1 5 10

WE CLAIM:

1. A bicyclic neurotrophin-derived peptide, or a functional equivalent thereof, capable of inhibiting a neurotrophin-mediated activity.

2. A bicyclic neurotrophin-derived peptide as defined in claim 1, comprising the internal reverse turn region of a neurotrophin.

3. A peptide comprising amino acids from positions 58-68 and 108-110 of a neurotrophin, wherein the amino acid from position 58 is covalently bound to the amino acid from position 108 and the amino acid from position 68 is covalently bound to the amino acid at position 110 to form a bicyclic structure, or a functional equivalent thereof.

4. A peptide as defined in claim 3, having the amino acid sequence of formula (I):

$$AA^{68}\text{-}XX\text{-}C^{58}\text{-}C^{108}\text{-}V\text{-}AA^{110} \qquad (I)$$

wherein

AA^{68} is selected from cysteine, desamino cysteine, and an N-terminally blocked cysteine;

AA^{110} cysteine, the descarboxylated form thereof, and a C-terminally blocked cysteine; and XX represents a peptide comprising from 1-10 amino acid residues.

5. A peptide as defined in claim 4, which inhibits NGF.

6. A peptide as defined in claim 5, having the amino acid sequence of formula (II):

$$AA^{68}\text{-}G\text{-}S\text{-}X_1\text{-}V\text{-}P\text{-}N\text{-}X_2\text{-}X_3\text{-}R\text{-}C^{58}\text{-}C^{108}\text{-}V\text{-}AA^{110} \qquad (II)$$

wherein

AA^{68} is selected from cysteine, desamino cysteine, and an N-terminally

blocked cysteine;

AA^{110} is selected from cysteine, the descarboxylated form thereof, and a C-terminally blocked cysteine; and

X_1 is an acidic amino acid;

X_2 is selected from the group consisting of a non-polar amino acid and an uncharged polar amino acid; and

X_3 is selected from the group consisting of an acidic amino acid and a non-polar amino acid.

7. A peptide as defined in claim 6, wherein

X_1 is selected from glutamic acid and aspartic acid;

X_2 is selected from proline and serine; and

X_3 is selected from aspartic acid and alanine.

8. A peptide as defined in claim 7, having the amino acid sequence:

C-G-S-E-V-P-N-S-A-R-C-C-V-C.

9. A composition comprising a peptide as defined in claim 1 in an amount effective to inhibit neurotrophin-mediated activity, and a suitable carrier.

10. A composition comprising a peptide as defined in claim 3 in an amount effective to inhibit neurotrophin-mediated activity, and a suitable carrier.

11. A composition comprising a peptide as defined in claim 4 in an amount effective to inhibit neurotrophin-mediated activity, and a suitable carrier.

12. A composition comprising a peptide as defined in claim 8 in an amount effective to inhibit neurotrophin-mediated activity, and a suitable carrier.

13. A method for inhibiting a neurotrophin-mediated activity comprising the step of exposing neuron cells to an effective amount of a composition as defined in claim 9.

14. A method for inhibiting a neurotrophin-mediated activity in a mammal comprising the step of administering to said mammal a therapeutically effective amount of a composition as defined in claim 9.

15. A method as defined in claim 14, wherein said composition is administered intraventricularly.

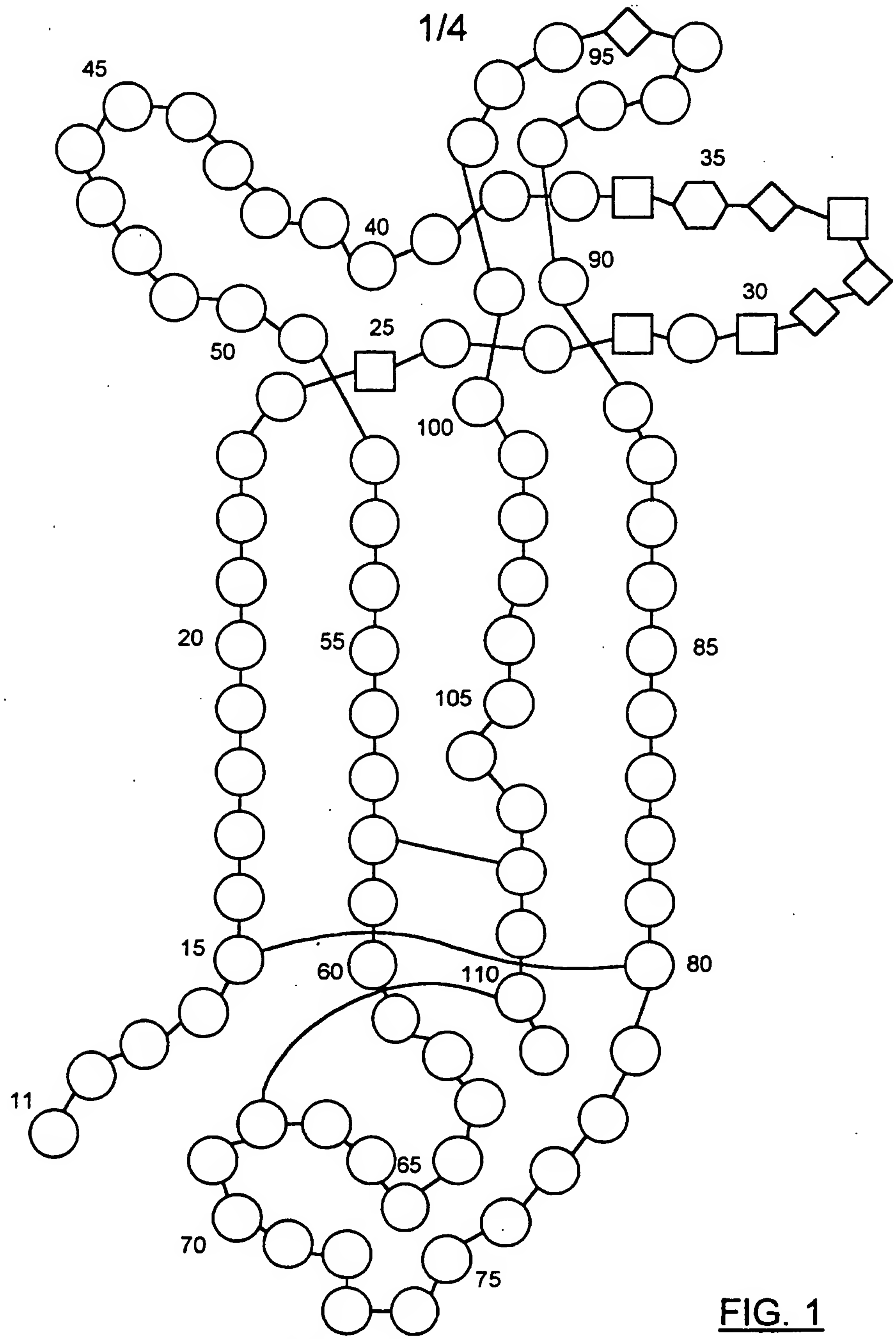

FIG. 1

LINEAR NGF PEPTIDE:

C^{68}-G-S-E-V-P-N-S-A-R-C^{58}-C^{108}-V-C^{110}

(SEQ ID NO:1)

CYCLIC NGF PEPTIDE:

C^{68}-G-S-E-V-P-N-S-A-R-C^{58}-C^{108}-V-C^{110}

(SEQ ID NO:1)

BYCYCLIC NGF PEPTIDE:

C^{68}-G-S-E-V-P-N-S-A-R-C^{58}-C^{108}-V-C^{110}

(SEQ ID NO:1)

FIG. 2

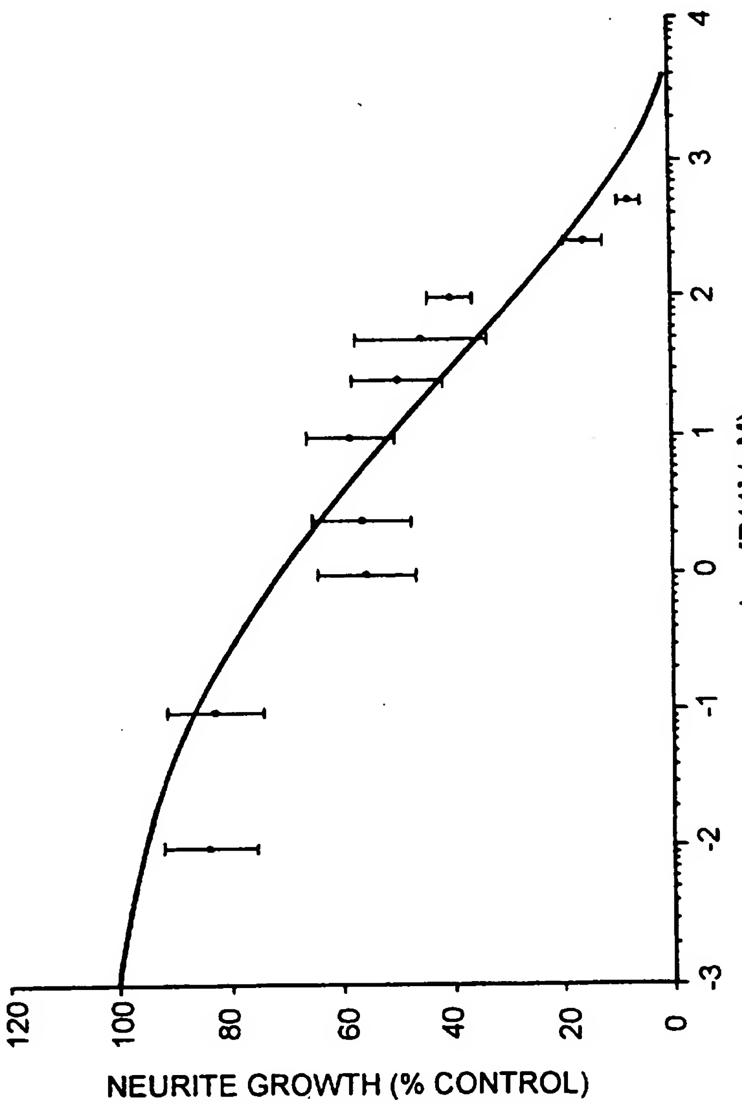
NEURITE GROWTH (% CONTROL)
120
100
80
60
40
20
0
Log [R11] (μM)
-3
-2
-1
0
1
2
3
4

FIG. 3

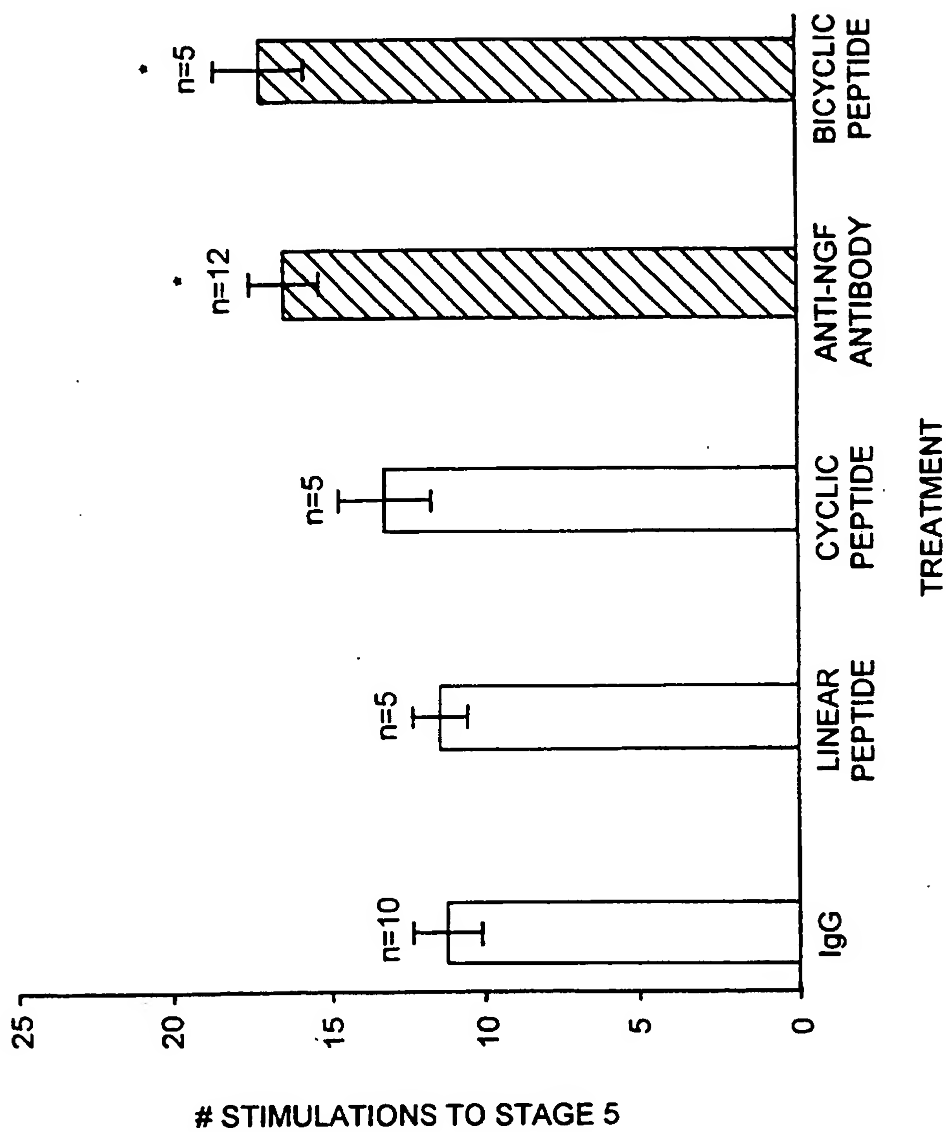
n=10
n=5
n=5
n=12
*
n=5
*
IgG
LINEAR PEPTIDE
CYCLIC PEPTIDE
ANTI-NGF ANTIBODY
BICYCLIC PEPTIDE
TREATMENT
25
20
15
10
5
0
STIMULATIONS TO STAGE 5

FIG. 4

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/CA 95/00603

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/475 C07K14/48 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,X	SOCIETY FOR NEUROSCIENCE ABSTRACTS, 24TH ANNUAL MEETING OF THE SOCIETY FOR NEUROSCIENCE, MIAMI BEACH, FLORIDA, USA, NOVEMBER 13-18, 1994, vol. 20, no. 1-2, 1994, page 678 XP002007155 K RASHID ET AL.: "Peptide mimics of an NGF domain inhibit kindling and neuronal sprouting in rats " see the whole document ---	1,2,9, 13-15

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
1 July 1996	12.07.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	**Authorized officer** Masturzo, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PC /CA 95/00603

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 92, no. 21, 10 October 1995, WASHINGTON US, pages 9495-9499, XP002007156 K RASHID ET AL.: "A nerve growth factor peptide retards seizure development and inhibits neuronal sprouting in a rat model of epilepsy" see the whole document ---	1,2,9, 13-15
A	WO,A,95 21193 (MC GILL UNIVERSITY) 10 August 1995 see the whole document -----	1-15

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA95/00603

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 13 - 15 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

..formation on patent family members

Interr ..al Application No
PC1/CA 95/00603

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9521193	10-08-95	AU-B- 1572695	21-08-95